

SUPPLEMENTARY FIGURE 1. ML-RR-cGAMP and ML-RR-cGAMP-D induce higher

frequency of antigen-specific T-cell responses than MPLA in WTB6 and *Ifnar*^{-/-} mice. Mice

were immunized twice with 20 μg of OVA in combination with ML-RR-cGAMP, ML-RR-

cGAMP-D or MPLA, each formulated in AddaVax; immune responses were evaluated 1 week

following the second immunization. Splenic antigen-specific IFN-γ responses induced by OVA

were measured by ELISpot upon stimulation with OVA CD4 peptide

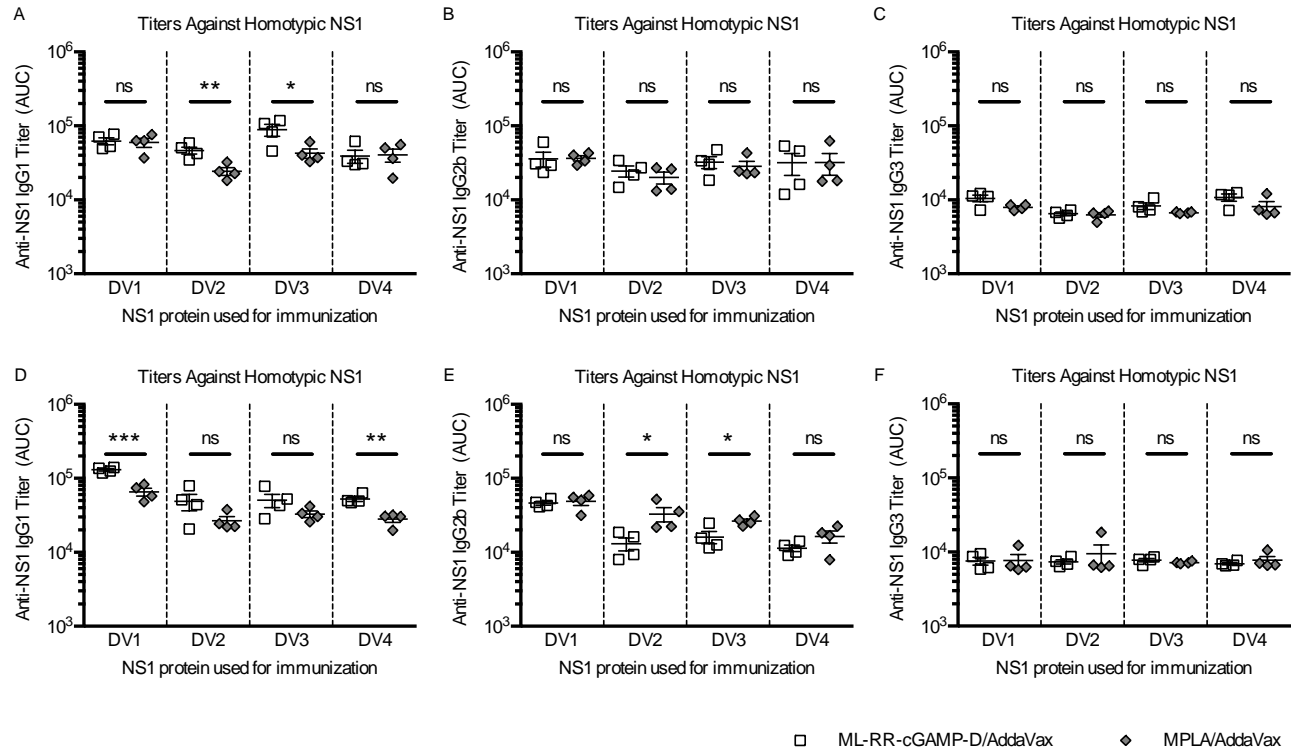
(₂₆₅TEWTSSNVMEERKIKV₂₈₀) or OVA CD8 peptide (₂₅₇SIINFEKL₂₆₄) in WTB6 (**A**) and *Ifnar*^{-/-}

(**D**) mice. The frequency of OVA-specific IFN-γ-, TNF-α- and IL-2-producing CD4⁺ and CD8⁺

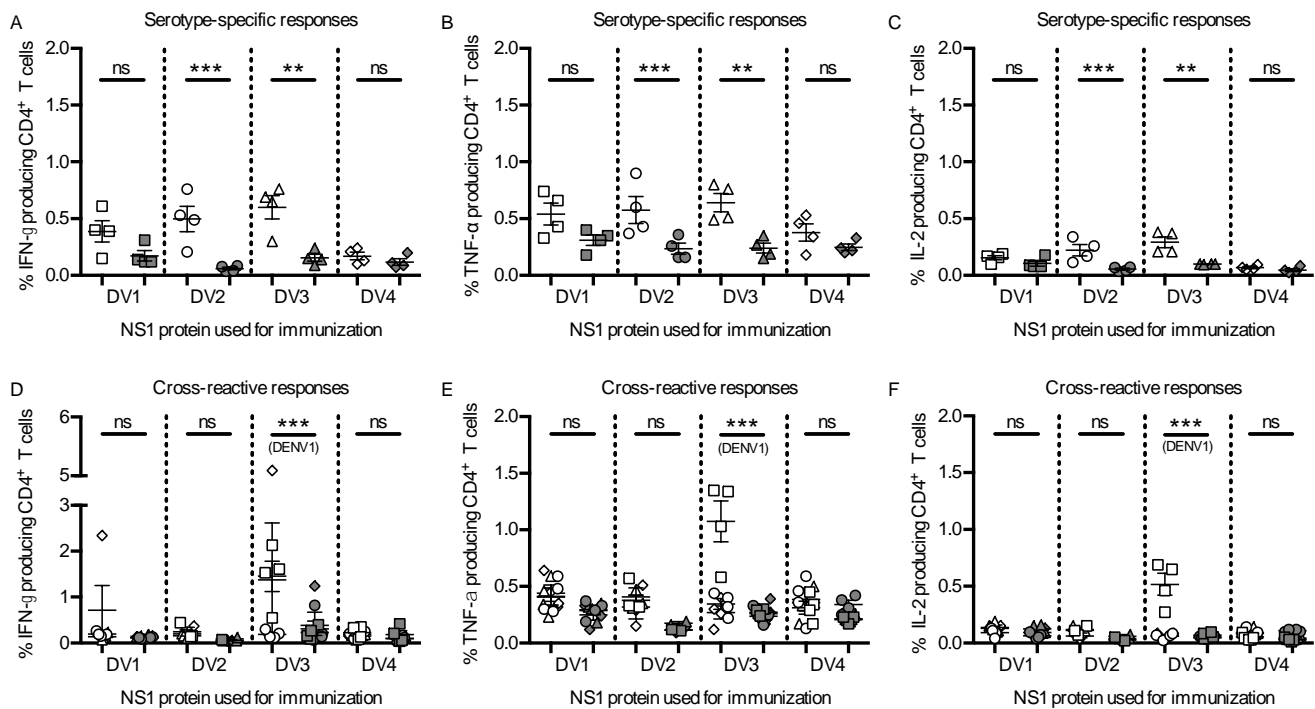
T cells in spleen were measured by ICS upon stimulation with OVA CD4 peptide and OVA CD8

peptide, respectively; responses were measured in WTB6 (**B-C**) and *Ifnar*^{-/-} (**E-F**) mice. Data are

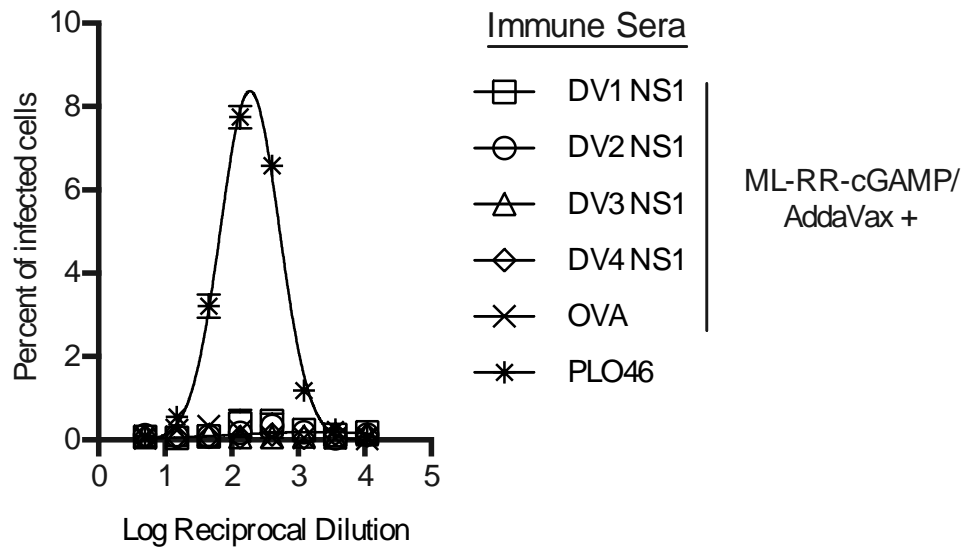
representative of 2 independent experiments (mean ± SEM; n=3; * p<0.05).



SUPPLEMENTARY FIGURE 2. ML-RR-cGAMP-D and MPLA induce comparable IgG subclass responses in WTB6 and *Ifnar*^{-/-} mice. Mice were immunized two times with 20 µg of each DENV NS1 serotype in combination with ML-RR-cGAMP-D or MPLA, each formulated in AddaVax. IgG1, IgG2b and IgG3 titers against homotypic NS1 proteins (i.e., against the same NS1 protein serotype used for immunization) were assessed by direct ELISA 1 week after the second immunization in WTB6 (A-C) and *Ifnar*^{-/-} (D-F) mice. Titers are shown as area under the curve (AUC) based on serially diluted serum samples. Data are representative from 2 independent experiments (mean ± SEM; n=4; * p<0.05, ** p<0.01, *** p<0.001).



SUPPLEMENTARY FIGURE 3. ML-RR-cGAMP-D elicits serotype-specific and cross-reactive anti-NS1 CD4⁺ T-cell responses in WT6 mice. T-cell responses in spleens from mice immunized twice with 20 μ g of each DENV NS1 serotype in combination with ML-RR-cGAMP-D (white symbols) or MPLA (gray symbols) formulated in AddaVax were assessed by ICS 1 week after the second immunization. Serotype-specific (**A-C**) and cross-reactive (**D-F**) IFN- γ , TNF- α and IL-2 production by CD4⁺ T cells was measured upon stimulation with NS1 proteins from DENV1 (square), DENV2 (circle), DENV3 (triangle) or DENV4 (diamond). Mean \pm SEM; n=4; * p<0.05, ** p<0.01, *** p<0.001.



SUPPLEMENTARY FIGURE 4. Immune sera induced by DENV NS1-ML-RR-cGAMP/AddaVax immunizations do not enhance *in vitro* DENV infection. DENV2 D220 was incubated with serially diluted pooled serum samples from *Ifnar*^{-/-} mice immunized with DENV NS1 or OVA in combination with ML-RR-cGAMP and used to infect K562 cells. Sera from mice immunized with DENV2 PLO46 were used as positive control. Data are plotted as the average of duplicate values, where the absolute percent infection of K562 cells is shown on the y-axis. Data are representative from 2 independent experiments